40 Leading Edge Essay
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Aging Research—Where Do We Stand and Where Are We Going?

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The 40th anniversary of *Cell* coincides with that of the National Institute on Aging (NIA). Indeed, *Cell* papers on NIA-funded research helped move the field into a genetic and molecular era. Now is a fair time to ask whether we are far down a trail leading to a deep understanding of aging or whether we are still tiptoeing cautiously at the trailhead.

There is a general perception that the effects of aging have somehow been slowed during our lifetimes. Memories and photographs of our grandparents when they were in their 60s look more like people in their 70s or even 80s today. To address more rigorously whether effects of aging have indeed been slowed, we must first define what we mean by aging. One metric comes from human actuarial data and from studies in lower laboratory organisms like yeast, worms, and flies—the average and maximum life spans. In mammalian systems, many more consequences of aging are readily measurable, such as functional decline in performance tests, deterioration of individual tissues, and degradation in broad measures of metabolic health. These latter measures define the health span of the organism, the maintenance of which I believe to be the most important goal of ongoing aging research. Are we making significant progress?

The magnitude of the challenge is illustrated by considering known causes of aging. The good news is that many mechanisms causing aging, as well as pathways that can mitigate effects of aging, have been identified. This is also the bad news—aging processes and pathways offering an ability to modify their effects (discussed below) are extremely complex. It is widely assumed that aging is a major risk factor for most late-onset diseases (cancer, cardiovascular disease, diabetes, neurodegenerative diseases, etc.), and therefore interventions directed at aging offer an opportunity to ameliorate all these diseases at once. Although this idea has attracted much attention, we must also consider that the complexities of aging processes likely exceed those of specific diseases, and the challenge of reigning in the global decline of cellular processes across many tissues will be large.

At the cellular level, aging induces many potentially interconnected defects, including DNA damage in the nucleus and mitochondria, mitochondrial dysfunction leading to increased production of reactive oxidative species (ROS) and decreased production of ATP, oxidative damage to proteins and other macromolecules in cells, protein misfolding and aggregation, protein glycation, the induction of proinflammatory cytokines, telomere shortening, and cell senescense (López-Otín et al., 2013) (Figure 1). These will impact mitotically active tissues over time by triggering stem cell depletion by senescence and apoptosis (e.g., intestinal stem cells, hematopoietic stem cells, mesenchymal stem cells, etc.), and postmitotic tissues by causing cellular dysfunction and loss (e.g., muscle, heart, and brain). Beyond tissue-autonomous aging, it is now clear that the brain helps govern aging of many organs (Satoh et al., 2013; Chang and Guarente, 2014), i.e., dysfunction in the hypothalamus will exert systemic effects leading to functional decline and damage to cells and organs.

Offering some degree of hope, over the past quarter century, numerous genetic pathways in model organisms have been identified that can confront at least some of the villains of aging (Figure 1). Before this era, the most prominent ideas were that aging was simply due to wear and tear and, more recently, to the accumulation of oxidative damage in cells. The first genetic pathway implicated in aging was found in *C. elegans*, where Tom Johnson showed that hypomorphic mutations in a single gene *age-1* could extend the life span (Johnson, 2013), and Cynthia Kenyon and Gary Ruvkun defined a genetic/molecular pathway involving insulin/insulin-like growth factor (IGF) signaling, which included *age-1* (Kenyon, 2010). Downregulation of this pathway extended the life span, but knocking it out entirely could be deleterious, and these effects are conserved in organisms ranging from worms to mice.

My own lab was also interested in aging, and in 1991, two entering graduate students, Brian Kennedy (now CEO of the Buck Institute) and Nicanor Austriaco (now a Franciscan priest and advisor to the Vatican) began studying the aging of yeast mother cells, which senesce after giving rise to 20–30 daughter cells. *SIR2* emerged from these studies as an important gene combating yeast mother cell aging, and it is noteworthy that three *Cell* papers marked the trail of this research (Kennedy et al., 1995; Smeal et al., 1996; Kennedy et al., 1997). In this case, the modest upregulation of yeast *SIR2* extended life span, but more prolific overexpression could be toxic. Indeed, a more recent genome-wide quantitative trait locus (QTL) analysis identified *SIR2* as the single most important yeast gene in determining the difference in mother cell life span between a lab strain and a clinical

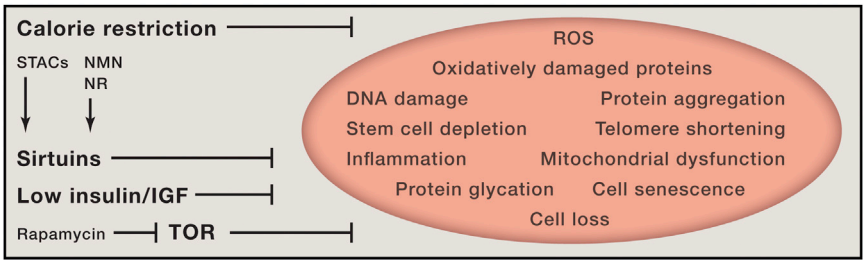

Figure 1. The Complexity of Aging
Shown is a cell under the forces of aging (causes depicted inside and mitigating pathways depicted outside). Possible small-molecule interventions are also indicated.

isolate (Stumpferl et al., 2012). *SIR2* orthologs have also been shown to extend life span in worms, flies, and mice (Guarente, 2013).

There was also a burning interest in that early period in what Sir2 protein's biochemical activity might be. One clue was that *SIR2* was associated with yeast genomic silencing, itself linked to the deacetylation of histones (Braunstein et al., 1996). But demonstrating any deacetylation activity by Sir2 protein in vitro initially proved impossible. Another clue came from a somewhat baroque study showing that Sir2 protein had a very weak ability to transfer ADP-ribose from NAD^+ to the irrelevant substrate bovine serum albumin in vitro (Frye, 1999). Through a series of serendipitous experiments, my then-postdoc Shin Imai and I were able to arrive at the conclusion that yeast Sir2 protein and its mammalian ortholog, SIRT1, were NAD^+-dependent protein deacetylases (Imai et al., 2000), thereby linking metabolism and aging. Sir2-related proteins are termed sirtuins, and in mammals, the seven sirtuin proteins are designated SIRT1–7. These seven proteins are all encoded by nuclear genes but mainly reside in discrete cellular compartments—the nucleus (SIRT1, SIRT6, and SIRT7), the cytosol (SIRT2), and the mitochondria (SIRT3, SIRT4, and SIRT5) (Verdin et al., 2010).

Metabolism and aging had already been linked in the 1930s by the diet termed calorie restriction (CR), which became the gold standard to slow aging and extend the life span in rodents. Many had assumed that CR worked by generally slowing down metabolism and the presumed accompanying oxidative damage in cells. But the fact that the first two longevity pathways described, insulin/IGF and sirtuins, both affected metabolism offered the possibility that CR might extend life span by altering specific pathways. For sirtuins, the data supporting this idea are many (Guarente, 2013). First, CR induces the levels and activities of several sirtuins. Second, knocking out any one sirtuin gene abolishes at least some of the phenotypes of CR, including extended life span (SIRT1). Third, transgenic mice overexpressing SIRT1 in brain or SIRT6 globally live longer and show a better metabolic profile and retention of tissue integrity as they age (Kanfi et al., 2012; Satoh et al., 2013). Fourth, many of the substrates deacylated and thus activated by sirtuins are precisely those mediating important physiological effects of CR, i.e., induction of oxidative mitochondrial metabolism and its accompanying stress resistance (Verdin et al., 2010; Guarente 2013). Fifth, SIRT1 activator compounds (STACs) have been identified by screening for molecules that activate the enzyme in vitro, and these include polyphenols and more specific novel chemical entities (Sinclair and Guarente, 2014). Importantly, STACs have been shown to extend life span in mice on the normal diet (Mercken et al., 2014), slow the progression to tissue dysfunction in mice and nonhuman primates (Mattison et al., 2014), and induce patterns of gene expression resembling CR (Guarente, 2013).

CR may be viewed as high up on the pyramid of health, with a potential to redress many of the cellular and tissue defects occurring during aging (Figure 1). However, there are major issues to consider before this presumption can be considered a certainty, at least as it might apply to humans. In particular, there is a paucity of data on the effects of CR on aging in primates. Two studies in nonhuman primates came to opposite conclusions as to whether CR extends life span (Colman et al., 2009; Mattison et al., 2012). Both studies, as well as short-term human studies (Redman et al., 2011), do agree that CR can improve metabolic parameters, such as glucose metabolism. Although it is possible that CR could extend human life span, it is also possible that it would not, if it also exerts negative effects that cancel the metabolic benefit (e.g., untoward effects on the immune system). Although difficult to conduct, more extended studies of the effects of CR on humans would be informative.

Another aspect of aging has recently emerged from the identification of NAD^+ as the sirtuin cosubstrate. We first suspected that changes in NAD^+ or the NAD^+/NADH ratio activate sirtuins and help drive effects of CR (Guarente, 2000), a finding later supported by experimentation (Guarente, 2013). What we did not anticipate are the more recent findings that NAD^+ levels appear to decline during aging across a broad spectrum of species (Ramsey et al., 2008; Mouchiroud et al., 2013). There are two main hypotheses to explain this decline (Figure 2). First, aging is associated with cumulative damage to nuclear DNA, and the chronic activation of the DNA repair enzyme poly-ADP-ribose polymerase (PARP) would consume NAD^+ as it ADP-ribosylates proteins at sites of damage (Bai et al., 2011; Mouchiroud et al., 2013). Second, the gene encoding the NAD^+ synthesis enzyme nicotinamide phosphoribosyl transferase (NAMPT) is activated by the circadian clock and is a major link between the clock and the diurnal staging of metabolic reactions (Nakahata et al., 2009; Ramsey et al., 2009). For example, fat oxidation in mitochondria is circadian due to pulsatile NAD^+ synthesis and SIRT3 activation, and knocking out SIRT3 disrupts this temporal organization (Peek et al., 2013). Because the amplitude of the central clock in the suprachiasmatic nucleus of the hypothalamus (and perhaps cell-autonomous clocks in other tissues) may decay during aging in mammals (Chang and Guarente, 2013), a decline in NAMPT from a reduction in the output of the circadian clock may result in a decline in NAD^+ synthesis with age.

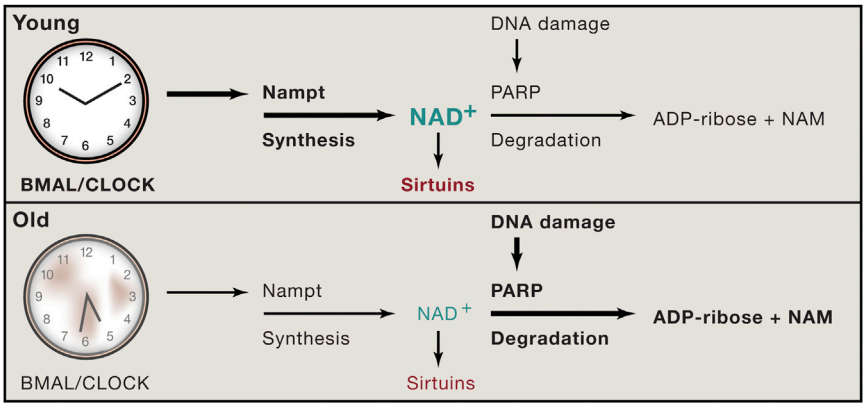

Figure 2. NAD+ Declines with Aging

Shown are two possible mechanisms of decline. The left depicts a reduction in synthesis due to a decline in the amplitude in the circadian clock with aging. The right depicts an increase in degradation due to chronic DNA damage and PARP activation with aging.

Importantly, this aging-induced decline in NAD^+ may be reversed in rodents by supplementing the diet with the NAD^+ precursors nicotinamide mononucleotide (NMN) (Ramsey et al., 2008; Yoshino et al., 2011) or nicotinamide riboside (NR) (Cantó et al., 2012). These compounds can ameliorate or even reverse metabolic defects in mice, in part by restoring proper stoichiometry in the expression of nuclear- and mitochondrially encoded mitochondrial proteins via SIRT1 reactivation (Gomes et al., 2013). It will be interesting to see whether combining NMN or NR with STACs exerts a synergistic effect in treating the decline associated with aging in rodents and primates.

Since 2000, other pathways have also been implicated in aging, including AMP kinase (AMPK) (Gowans and Hardie, 2014), which is upregulated when energy is limiting, and target of rapamycin (TOR) (Shimobayashi and Hall, 2014), a nutrient sensor that must be downregulated, by rapamycin, for example, for beneficial effects. It is now clear that these pathways and the insulin/IGF and sirtuins pathways all interact in mammals to regulate a wide swath of metabolism in response to diet and also trigger mechanisms to relieve oxidative stress. Accordingly, these pathways also regulate the functionality of mitochondria. For example, downregulation of TOR stimulates mitophagy to improve mitochondrial quality, and upregulation of AMPK and sirtuins, partners in a mutually reinforcing feedback loop, activates many aspects of mitochondria, including biogenesis, oxidative metabolism, and an accompanying oxidative stress resistance. However, it is still not clear how many of the vast number of degenerative changes in aging can be influenced by this network of metabolic pathways.

With regard to intervening pharmacologically in the above pathways, one must be cautious in concluding that modulating any of them would be an effective way to mitigate effects of aging. For example, downregulation of mTOR by rapamycin extends murine life span (Shimobayashi and Hall, 2014). But an exhaustive examination of the tissues of treated mice compared to untreated controls showed that, in some tissues, rapamycin indeed preserved integrity in old animals, whereas in others, it had no effects, and in still others, it had negative effects (Neff et al., 2013). It remains possible that at least some of the effect on life span by rapamycin in mice is due to the slowing of cancer. Likewise, blockade of the IGF1 receptor in humans would run the risk of sarcopoenia and poor cardiac health because muscle and heart depend on IGF1 signaling for their maintenance. In addition, blockade of the insulin receptor would trigger insulin resistance in muscle, liver, and adipose tissue. SIRT1 activation may actually aid the growth of certain preexisting tumors, perhaps due to its deacetylation and downregulation of p53 (Guarente, 2013), although in other cases, it seems to repress the initiation of tumors. The expression of SIRT3 and SIRT6, in contrast, has also been associated with beneficial effects that overlap CR, including suppression of tumor growth. It might be an important addition to the tool kit of health span interventions if small-molecule activators of these latter two sirtuins can be found.

Fascinating, newer approaches to study aging are also emerging and may yield new strategies for intervention. Parabiosis is a classic procedure in which the circulations of two mice are connected surgically. By thus fusing genetically identical old and young mice, some of the effects of aging in an old mouse can be ameliorated, a finding that would please Bram Stoker (Conboy et al., 2013). This approach has already led to the identification of specific molecules from the young mouse, which appear to rejuvenate aspects of aging of the old mouse (Katsimpardi et al., 2014). How such circulating molecules identified by parabiosis might relate to the metabolic pathways mentioned above is not clear at this point. Another current approach in aging research is to study centenarians (people 100 years old or older), and especially families in which centenarians cluster significantly (Sebastiani and Perls, 2012; Milman et al., 2013). The idea is to identify genetic factors that explain the extreme longevity of these rare individuals. This approach would seem to hold considerable promise, but we must keep in mind that identical twin studies suggest that genes account for only about 25% of human life span.

In addition, we may have but scratched the surface of what bioinformatics can provide in identifying new genes and pathways important in human aging, as well as allowing for the knowledge we have already gained to be applied in a more effective, personalized way. Analysis of the transcriptome, epigenome, and proteome of individuals spanning a wide age range will provide the most detailed phenotyping of human aging so far. For example, it has already been possible to conduct transcriptome analysis of cortical regions obtained from human brains of different ages preserved at autopsy (Lu et al., 2004). This has showed that a small fraction of genes change in their transcription levels in a characteristic way correlated with the

age of the subjects. Plotting these transcript levels (molecular aging) versus chronological age at time of death thus yields a straight line for this set of genes. Within a large enough cohort, there ought to be outliers for whom the molecular age significantly deviates from the chronological age; i.e., they would show slower or faster transcriptional changes in many or all of the genes sensitive to aging. Further analysis of these outliers for enrichment in SNPs defining specific haplotypes may shine a light on genes and pathways that favor slow or rapid aging (Glorioso et al., 2011). If SNPs passing significance for genome-wide discovery prove difficult to find, analyses of transcriptional (or epigenetic) differences in the outliers (e.g., unusually high expression of gene X in all slow aging brains) may give clues about genes and pathways that determine slow or rapid aging. This latter approach also has the advantage of identifying mechanisms that are not genetically based.

If the genes and pathways that seem to correlate with slow or fast aging can be thus identified by big data analysis, resulting hypotheses about brain aging may be tested by conducting field studies. Possible treasure troves are the various long-term human longitudinal studies, which provide a cornucopia of health information spanning decades and, in some cases, provide access to genotyping. This may potentiate testing whether genetic haplotypes that correlate with slow or rapid aging identified bioinformatically exert predictable effects in a human population over the course of a lifetime. For example, one might test whether haplotypes correlating with slow molecular brain aging protect against cognitive decline or neurodegenerative diseases in these longitudinal studies.

For all diseases, there is a need to use haplotype data to stratify people by their DNA sequence to determine the best treatment options, i.e., personalized medicine. Such stratification would also guide the selection of which humans to choose for clinical trials of potential new drugs. In the context of aging, extending bioinformatics studies on brain aging to other tissues may reveal whether relevant haplotypes affect all tissues, or, more likely, affect aging in some tissues but not others. Tissue-specific haplotypes may overlap those associated with susceptibility to late-onset diseases impacting those same tissues. Because drugs may affect different tissues in different ways (e.g., rapamycin), this information would guide application of the right drug for the right person. This would be formally analogous to genotyping tumors to identify markers, which will then guide therapy.

Finally, IPS technology may also become important in combating effects of aging (Sánchez Alvarado and Yamanaka, 2014). If specific tissues vulnerable to aging in individuals can be identified bioinformatically, then differentiated cells generated by IPS technology from that person may potentiate drug screening to protect those cells from stressors and provide an avenue toward new therapeutics. Beyond this approach, one can imagine correcting the offending haplotype in IPS cells by CRISPR/Cas9 technology and reintroducing genetically reprogrammed cells into the patient, an approach that has been widely discussed for frank diseases (Hsu et al., 2014). Unfortunate tissues bearing haplotypes that cause rapid aging may thus be rescued by early interventions even before the onset of significant physiological decline. Although IPS-based approaches appear promising, they face many hurdles, including the successful incorporation of new cells into existing organs.

In closing, the past two centuries have witnessed advances at many levels that allow people to live longer and more productive lives. I have attempted to place current research on the biology of aging into this context and have arrived at a few predictions. First, it will be more achievable and desirable to extend human health span rather than life span per se. Yes, a historical perspective would indicate that average life span has been increasing in the developed world for more than a century, and this trend has not abated. Much of this increase is due to improved sanitation and reduction of insect-borne diseases, reduced mortality of infants, children, and birthing mothers and advances in medicine including vaccines, antibiotics, and antiviral drugs. Epidemiological studies and medical research targeted at specific diseases has also continued to push out average survival. On the other hand, certain environmental factors may be placing these gains at risk, such as diet-induced obesity leading to diabetes and pollution leading to reduced air and water quality, as well as global warming. Changes in maximum human life span will, in my opinion, be quite difficult to achieve and will take many years to even assess. From the point of view of economic and societal benefits, striving to make people healthier longer without necessarily extending their maximum life span may be the wisest course. Put another way, the nightmare scenario would be to extend maximum human life span without extending health span.

Second, bioinformatics will play a substantial role in the progress of aging research, especially as it applies to humans. There may already be buried in the sea of ever-increasing human genomic data novel clues about genes and pathways that govern aging in different tissues. In this regard, it remains to be seen how much of aging will prove to be systemic and affect all tissues simultaneously emanating from brain signals, for example, and how much will be tissue autonomous.

Third, aging and the genes and pathways that govern its effects are complex. It is not likely that there will be a silver bullet for aging any more than there will be a silver bullet for cancer. However, there will likely be novel pharmaceutical interventions for the effects of aging emerging directly from aging research. These interventions may need to be tissue specific, taking into account the personalized way aging impacts an individual tissue-by-tissue. Overall, it is an exciting, albeit uncertain, time to speculate how human health will be impacted in the decades to come by research on the biology of aging.

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